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DISTRIBUTION OF AERIALLY APPLIED *BACILLUS THURINGIENSIS* FOR CONTROLLING DEFOLIATING INSECTS IN A HARDWOOD FOREST

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Abstract

Both Dipel® *and Thuricide*® (Bacillus thuringiensis *Berliner) water-base spray formulations were aerially applied at a rate of 2 gal/acre to the semiclosed canopy of an oak forest in New York State to develop improved methods for controlling the gypsy moth.*

Techniques have been developed for quantifying spray deposit directly on hardwood foliage. No statistical differences were apparent between Dipel and Thuricide in mean drop-size spectra, deposit coverage of the forest canopy, and deposit distribution at the forest floor. The average volume median diameter of Dipel formulation recovered along subplot transects was 335 µm; that of Thuricide, 351 µm. On the average, about 10 percent of the spray deposit was recovered from the upper oak crowns, 8 percent from the lower crowns, 9 percent from under the sample trees at forest floor level, and 9 percent along the subplot transect lines at the forest floor level. The mean number of drops per square centimeter of foliage was six on the upper oak crowns, four on lower crowns, five under the trees, and five on Kromekote® *cards along the deposit sampling lines. A higher deposition of spray in the forest canopy is needed for increased effectiveness of insect control.*

KEYWORDS: *Insect damage control (hardwood forest), defoliation damage, spraying (-pest control.*

INTRODUCTION

Environmentally acceptable *Bacillus thuringiensis* Berliner (*B.t.*) offers a potential for controlling forest defoliating insect pests (Tripp 1972, Smirnoff et al. 1973, Lewis et al. 1974, Maksymiuk and Orchard 1974). Success or failure of aerial application of insecticides, such as *B.t.*, and insect viruses depends on adequate and uniform coverage of tree crowns with spray deposit.

Quantitative information on distribution of spray deposit for the oil-based chemical insecticide (Zectran[1]) has been recently developed.[2] Lewis and Connola (1966) emphasized the need for similar information to better evaluate use of *B.t.* to control the gypsy moth (*Porthetria dispar* (Linnaeus)).

A study was conducted to obtain information on spray deposit distribution as part of the field experiment to control the gypsy moth with Dipel® and Thuricide® *B.t.* water-base spray formulations in the State of New York in 1972 (Lewis et al. 1974).

This paper describes developed techniques and presents results on the drop-size spectra and the quantitative vertical and horizontal spray deposit distribution of Dipel and Thuricide in the hardwood forest canopy and at the forest floor level.

MATERIALS AND METHODS

Test Area

The experimental plots were located in the mountainous terrain on ridgetops south of Howes Cave, New York. Predominant tree species were chestnut oak (*Quercus prinus* L.), white oak (*Q. alba* L.), and northern red oak (*Q. borealis* Michx. f.). These trees, from 5 to 8 inches (in) in diameter, comprised a semiclosed forest canopy.

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[1] *Mention of products and companies by name does not constitute endorsement by the U.S. Department of Agriculture, nor does it imply approval of a product to the exclusion of others which may also be suitable.*

[2] *Bohdan Maksymiuk, John Neisess, R. A. Waite, and R. D. Orchard. Distribution of aerially applied mexacarbate in a coniferous forest and relationship to mortality of western spruce budworm. Unpublished data on file at Forestry Sciences Laboratory, Corvallis, Oregon, 1974.*

Formulations

Two preparations of *B.t.* were used. Dipel formulation contained: Dipel, a wettable powder (Abbott Laboratories, North Chicago, Ill.)--1 pound (lb); Cargill's Insecticide Base (Cargill, Inc., Minneapolis, Minn.)--2 quarts (qt); Chevron Spray Sticker (Chevron Chemical Co., San Francisco, Calif.)--6 fluid ounces (oz); and water--to make 2 gallons (gal). Thuricide (an emulsifiable concentrate) formulation contained: Thuricide-HPC (Sandoz, Inc., Homestead, Fla.)--2 qt; Cargill's Insecticide Base--2 qt; Chevron Spray Sticker--6 fl oz; water--to make 2 gal. In each of two spray applications, 2 gallons per acre of each formulation, containing *B.t.* equivalent to about 8 billion International Units of potency,were used.

Fluorescent tracers that absorb and fluoresce at different wavelengths were added to the spray formulations for quantitative assessment of spray deposit. Brilliant Sulphoflavine FFA was used for the first application, and Rhodamine B. Extra S (both from General Aniline and Film Corp., New York, N.Y.) was used for the second application (0.1-percent-weight-per-volume concentration).

Aerial Application

The physical layout was identical for both Dipel and Thuricide treatments. Two 80- to 100-acre plots were treated twice with Dipel and two with Thuricide. Each of the four test plots contained five 0.1-acre subplots (fig. 1). The first spray application was made when oak leaves were about half-grown. The second application was about 10 days later. The insecticide treatments for this study were assumed to be a completely random assignment to the four plots. Sides of the trees and applications were handled as split plot factors of the chemical whole plot treatments. The relationship between top and sides was analyzed for each plot using subplots as replications.

Estimated windspeed during applications ranged from 1 to 10 miles per hour (mi/h). Spray was applied by a fixed-wing aircraft (Grumman AgCat) equipped with four Beecomist® spinning nozzles (Beeco Products Co., Fort Washington, Pa.). The spray system was calibrated to deliver 2 gal per acre (18.71 liters/hectare) for 100-foot (30.48-meter) swath width. Spraying speed was about 85 mi/h (38 m/sec) and spraying height was about 45 ft (13.71 m) above forest canopy. Separate spray deposit assessments were made on the four plots after each of the two applications.

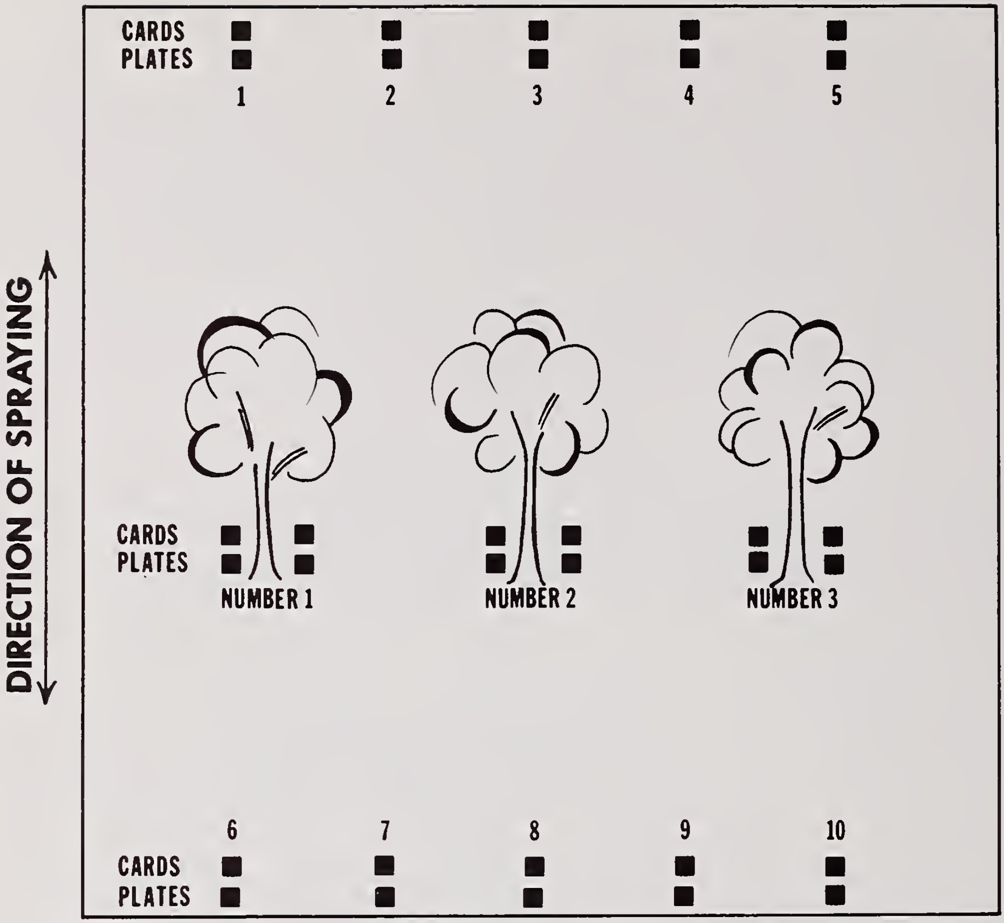

Figure 1.--Subplot layout showing sampling trees for determining spray deposit coverage of the tree crowns, and pairs of Kromekote cards with aluminum plates for assessing spray deposit at the forest floor level.

Drop-Size Spectra

The volume median diameter[3/] was determined by methods developed by Maksymiuk and Moore (1962) and Maksymiuk (1964). Kromekote® cards, supported by wire cardholders (Maksymiuk 1959), were placed along sampling lines and under sampling trees to sample deposit for determining vmd (fig. 1).

[3/] *Volume median diameter (vmd) is the diameter of a drop which satisfies the requirement that half the volume of liquid be in drops smaller than itself and half in drops larger.*

Spray Deposit Coverage of Tree Crowns

Each subplot contained three oak trees for sampling deposit on tree crowns. Oak foliage samples were used for determining spray deposit in gallons per acre and number of spray drops per square centimeter. Immediately after the spraying, foliage samples were collected from the upper (top) and lower crowns from the four sides (north, south, west, and east) of each of the three sample trees in each subplot. About 25 leaves were taken from each crown level. This foliage was carefully examined to exclude any insects. Then the samples were placed in paper bags which were sealed and labeled. All samples were shipped to the Forestry Sciences Laboratory in Corvallis, Oregon, for spray deposit analysis.

For determining the deposit on foliage in gallons per acre, 51 disks, 1 centimeter (cm) in diameter, were cut out of each crown sample of randomly selected leaves. Before the disks were cut, foliage samples were placed overnight in the refrigerator to absorb moisture, thereby reducing brittleness. The disks were cut from different locations on the individual leaves to account for the variability of deposit distribution. The samples, representing 40 cm^2 of the foliage area, were used for spray deposit assessment.

The deposit was eluted with distilled water from the 40 cm^2 of foliage disks. The amount of fluorescent tracer in the wash was determined by Turner spectrofluorometer, Model 430. The quantity of tracer for each sample was obtained from a previously developed standard curve showing the linear relationship between the amount of fluorescent tracer and fluorometer reading. Data were adjusted for the natural fluorescent background of the foliage samples, obtained shortly before the application of *B.t.* and for percent recovery of the fluorescent material from oak foliage, then converted to gallons per acre. This technique was similar to that described by Maksymiuk et al. (see footnote 2) for quantifying deposit on coniferous foliage, adapting the general principles reported by Yates and Akesson (1963).

Similarly, for determining number of spray drops, 51 disks, 1 cm in diameter, representing 40 cm^2 of leaf, were used. The spray drops containing fluorescent tracer were counted under a microscope illuminated by reflected ultraviolet light.

Spray Deposit Distribution at the Forest Floor

Aluminum plates (6 by 6 in) and white Kromekote cards (4 by 5 in) were positioned under the sample tree canopies and along the subplot transect lines at right angles to the direction of the spraying aircraft.

Two aluminum plates and two Kromekote cards were positioned under the canopy of each sample tree. The quantity in gallons per acre of the spray deposit recovered on aluminum plates at ground level under the tree canopies was determined by fluorometric analysis. The deposit on the plates was eluted with distilled water, read in a spectrophotometer, compared with a previously developed reference curve, and then corrected for percent recovery of the fluorescent material.

The number of drops was determined from the Kromekote cards by counting the drops in a 4-cm^2 central area of each Kromekote card, under a dissecting microscope illuminated by ultraviolet light.

RESULTS AND DISCUSSION

Drop-Size Spectra

Data are summarized in table 1. Based on analysis of variance, no statistically significant differences were detected among any of the mean vmd's compared. The variation in the drop-size spectra can be attributed to the differential screening effect by foliage on deposit (Maksymiuk 1963a), the variation in performance of the Beecomist spinning nozzles, and the presence of plant fibers in the spray formulation traceable to the molasses used in the Cargill Insecticide Base.

Spray Deposit Coverage of Tree Crowns

Dipel and Thuricide spray deposits on oak trees are shown in tables 2 and 3. The mean deposit, both in gallons per acre and number of drops per square centimeter, was higher for Thuricide than for Dipel treatments. However, this difference was not large enough to be detected as significant ($P<0.05$) when tested by analysis of variance.

The horizontal spray deposit distribution on the four sides of the lower portions of the tree crowns is given in table 2. No statistically significant differences were detected between the mean Dipel and Thuricide deposits for the first, second, and combined applications.

The deposits for Dipel and Thuricide were combined to obtain a larger sample size for testing reproducibility of spray applications. The mean Dipel and Thuricide spray deposit for the first spray application (0.202 gal/acre) was higher than the mean deposit for the second application (0.103 gal/acre). This mean difference was highly significant ($P<0.009$). It can indicate the need to increase the accuracy of aerial application for more reproducible field treatments. No statistical differences were detected in the mean deposit expressed in the number of drops per square centimeter.

Vertical spray deposit distribution is presented in table 3. Amounts of spray deposited on the upper and the lower portions of the tree crowns are compared. Consistently, more spray was deposited on the upper than

Table 1.--*Drop-size spectra of* Bacillus thuringiensis *formulations*

Plot	Dipel			Plot	Thuricide		
	Application number	Along transects	Under trees		Application number	Along transects	Under trees
		- - - - - - - - - - *Volume median diameter, μm* - - - - - - - - - -					
A	1	[1] 296±119	--	C	1	414±114	--
A	2	374±161	--	C	2	249±47	--
B	1	376±117	437±233	D	1	395±64	279±92
B	2	294±222	283±286	D	2	346±104	316±128
Mean		335±59	360±150	Mean		351±44	298±66

[1] Confidence intervals are given at the 95-percent probability level.

Table 2.--*Comparison of mean spray deposit on the sides of the tree crowns*

Crown side	Application number	Dipel[1]		Thuricide[2]		Dipel + Thuricide	
		Gallons per acre	Drops per square centimeter	Gallons per acre	Drops per square centimeter	Gallons per acre	Drops per square centimeter
North	1	0.186	1.6	0.249	6.5	0.218	4.0
	2	.057	2.3	.141	3.4	.099	2.8
	1+2	.122	2.0	.195	5.0	.158	3.4
South	1	.219	1.5	.174	7.0	.196	4.2
	2	.083	2.8	.115	3.6	.099	3.2
	1+2	.151	2.2	.144	5.3	.148	3.7
East	1	.153	1.5	.201	6.2	.177	3.8
	2	.075	2.4	.136	3.7	.106	3.0
	1+2	.114	2.0	.168	5.0	.141	3.4
West	1	.193	1.7	.241	6.0	.217	3.8
	2	.070	2.6	.144	3.7	.107	3.2
	1+2	.132	2.2	.192	4.8	.162	3.5
Mean of four sides	1	.188	1.6	.216	6.4	.202	4.0
	2	.071	2.5	.134	3.6	.103	3.0
	1+2	.130	2.0	.175	5.0	--	--

[1] Plot A + B.

[2] Plot C + D.

Table 3.--*Comparison of mean spray deposit on the tops and sides of tree crowns*

Tree crown	Application number	Dipel[1]		Thuricide[2]		Dipel + Thuricide	
		Gallons per acre	Drops per square centimeter	Gallons per acre	Drops per square centimeter	Gallons per acre	Drops per square centimeter
Top	1	0.204	1.5	0.209	14.7	0.206	8.1
Sides		.169	1.0	.151	11.1	.160	6.0
Top	2	.112	1.9	.286	6.4	.199	4.2
Sides		.071	1.2	.169	3.9	.120	2.6
Top	1+2	.158	1.7	.252	10.6	.205	5.9
Sides		.120	1.1	.158	7.5	.139	4.0
Top + sides[3]	1	.186	1.2	.180	12.9	.183	7.0
Top + sides	2	.091	1.6	.228	5.2	.160	3.3
Top + sides	1+2	.139	1.4	.204	9.0	--	--

[1] Plot B.

[2] Plot D.

[3] Statistical tests were not made for marginal means because plots were not replicated for assessing deposit on treetops.

the lower portions of the tree crowns. However, because of deposit variation among subplots, the differences between crown levels were not statistically significant for the Dipel and Thuricide treatments for the first, second, and combined applications except for the second application of the Thuricide treatment. In this case, the mean spray deposit for the combined applications (0.252 gal/acre) on the upper tree crowns was significantly higher compared with the mean deposit (0.158 gal/acre) on the lower crowns ($P<0.044$). Interaction between insecticides and crown sides was not significant, either for deposit in gallons per acre or for number of drops per square centimeter.

Spray Deposit Distribution at the Forest Floor

Spray deposits recovered under sampling trees and along sampling transects for both applications of Dipel and Thuricide are presented in table 4. Because of a lack of replication, no statistical analysis of the data was made, except for spray deposit along transects expressed in drops per square centimeter. Based on the analysis of variance, no statistical differences were detected between the mean spray deposits of Dipel and Thuricide, between applications, and interaction between insecticides and applications for the deposit under the sampling trees and along the transects (table 4).

Table 4.--*Comparison of spray deposit distribution of* Bacillus thuringiensis *at forest floor level*

Plot	Dipel					Plot	Thuricide				
	Application number	Under trees		Along transects			Application number	Under trees		Along transects	
		Gallons per acre	Drops per square centimeter	Gallons per acre	Drops per square centimeter			Gallons per acre	Drops per square centimeter	Gallons per acre	Drops per square centimeter
A	1	--	--	--	3.3±1.4	C	1	--	--	--	6.0±3.2
A	2	--	--	--	4.1±0.6	C	2	--	--	--	4.6±2.0
B	1	[1]0.117±.063	3.8±1.1	0.137±.088	4.1±1.3	D	1	0.189±.130	10.8±5.1	0.215±.147	10.8±4.8
B	2	.199±.412	1.8±1.0	.196±.232	1.6±0.9	D	2	.236±.169	4.0±2.2	.198±.110	5.0±1.9
Mean		.158±.207	2.8±0.6	.167±.133	3.3±0.3	Mean		.212±.125	7.4±3.4	.206±.113	6.6±0.6

[1] Confidence intervals are given at the 95-percent probability level.

If we assume a uniform application rate of 2.0 gal/acre, only 7.9 percent of the Dipel spray was recovered under sampling trees and only 8.4 percent along subplot deposit sampling lines. For Thuricide, 10.6 percent of deposit was recovered under the sampling trees, and 10.3 percent along subplot transects.

The direct method of sampling spray deposit on foliage for correlating it with insect mortality (Maksymiuk et al., see footnote 2) has an advantage compared with the indirect methods of sampling spray deposit reaching ground level (Maksymiuk 1963b). The impingement of spray drops can be affected by the physical, morphological, and biological characteristics of the particular sampling surface, associated airmass movements, and electrostatic charges. Therefore, the spray deposit should be sampled directly on the foliage where insects are feeding for determining dose-response relationships.

In conclusion, our techniques for sampling and quantifying spray deposit were satisfactory. Improved aerial application methodology is needed for obtaining more uniform and higher deposit coverage of the forest canopy. This can be achieved by combining more-suitable spray formulations and atomizing devices for desirable drop-size spectra and by increasing the accuracy of aerial applications.

METRIC CONVERSIONS

1 inch = 2.54 centimeters
1 gallon = 3.785 liters
1 fluid ounce = 29.57 milliliters
1 pound = 453.59 grams
1 quart = 0.9463 liters
1 foot = 0.3048 meters
1 gallon per acre = 9.354 liters/hectare
1 mile per hour = 44.704 centimeters per second

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